Micro-fabrication of three dimensional pyrolysed carbon microelectrodes

Hemanth, Suhith; Keller, Stephan Sylvest; Caviglia, Claudia; Amato, Letizia

Publication date:
2017

Document Version
Publisher's PDF, also known as Version of record

(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
30 March 2017 (30.03.2017)

WIPO | PCT

(10) International Publication Number
WO 2017/050808 A1

(51) International Patent Classification:
G03F 7/00 (2006.01) *G03F 7/213* (2006.01)
G03F 7/20 (2006.01) *G03F 7/38* (2006.01)

(21) International Application Number:
PCT/EP2016/072388

(22) International Filing Date:
21 September 2016 (21.09.2016)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
15186066.5 21 September 2015 (21.09.2015) EP

(71) Applicant: **DANMARKS TEKNISKE UNIVERSITET** [DK/DK]; Anker Engelunds Vej 101 A, 2800 Kgs. Lyngby (DK).

(72) Inventors: **HEMANTH, Suhith**; Gl. Holtevej 108B, 2840 Holte (DK). **KELLER, Stephan Sylvest**; Sunesvej 12, 3500 Værløse (DK). **CAVIGLIA, Claudia**; Stockholmsgade 19, 5. tv., 2100 Copenhagen Ø (DK). **AMATO, Letizia**; Nærumgade 1, 2.th, 2200 Copenhagen NV (DK).

(74) Agent: **GUARDIAN IP CONSULTING I/S**; Diplomvej, Building 381, 2800 Kgs. Lyngby (DK).

(81) Designated States *(unless otherwise indicated, for every kind of national protection available)*: AE, AG, AL, AM, AO, AT, AU, AZ, BA, BB, BG, BH, BN, BR, BW, BY, BZ, CA, CH, CL, CN, CO, CR, CU, CZ, DE, DK, DM, DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IR, IS, JP, KE, KG, KN, KP, KR, KW, KZ, LA, LC, LK, LR, LS, LU, LY, MA, MD, ME, MG, MK, MN, MW, MX, MY, MZ, NA, NG, NI, NO, NZ, OM, PA, PE, PG, PH, PL, PT, QA, RO, RS, RU, RW, SA, SC, SD, SE, SG, SK, SL, SM, ST, SV, SY, TH, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, ZA, ZM, ZW.

(84) Designated States *(unless otherwise indicated, for every kind of regional protection available)*: ARIPO (BW, GH, GM, KE, LR, LS, MW, MZ, NA, RW, SD, SL, ST, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, RU, TJ, TM), European (AL, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HR, HU, IE, IS, IT, LT, LU, LV, MC, MK, MT, NL, NO, PL, PT, RO, RS, SE, SI, SK,

[Continued on next page]

(54) Title: MICRO-FABRICATION OF THREE DIMENSIONAL PYROLYSED CARBON MICROELECTRODES

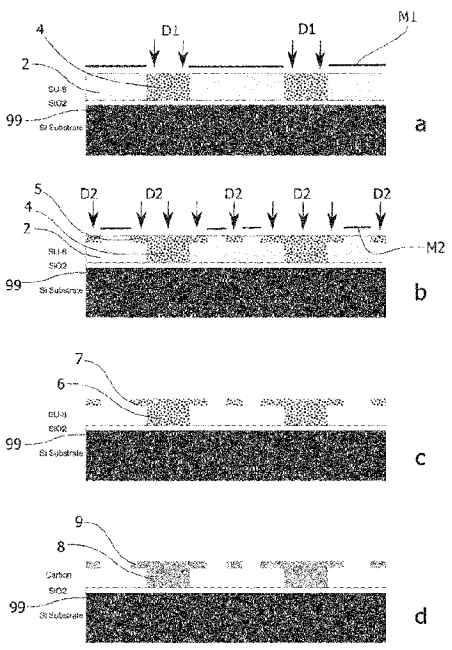


Fig. 1

(57) Abstract: The present invention relates in one aspect to a method of producing a three-dimensional microscale patterned resist template for a pyrolysed carbon microelectrode structure by means of UV-lithography. Coating a planar substrate with an epoxy-based negative photoresist, such as an SU-8 photoresist; soft baking the photoresist layer; performing a full depth exposure with UV light through a first mask; performing a partial depth exposure with UV light through a second mask; wherein the full depth exposure and the partial depth exposure are aligned to ensure that the first and second latent images are connected to each other; post-exposure baking the photoresist layer; and developing the microscale patterned resist template as a free-standing structure of cross-linked resist with lateral hanging structures that are supported by vertical support structures at a free height above the substrate. The method is characterized by a soft baking temperature below 70 °C. Repetitive coating and partial depth exposure allows for the fabrication of multiple level laterally interconnected structures. Carbonization of the resist template provides truly three-dimensional carbon microelectrode structures.

WO 2017/050808 A1

SM, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, KM, ML, MR, NE, SN, TD, TG).

Published:

— *with international search report (Art. 21(3))*

Micro-fabrication of three dimensional pyrolysed carbon microelectrodes

The present invention relates in one aspect to a method of producing a three-dimensional microscale patterned resist template for a pyrolysed carbon microelectrode structure by means of UV-lithography. In a further aspect, the present invention relates to a method of fabricating a three-dimensional pyrolysed carbon microelectrode structure by means of UV-lithography and subsequent carbonization. In a yet further aspect, the invention relates to a three-dimensional pyrolysed carbon microelectrode structure fabricated by the method.

BACKGROUND OF THE INVENTION

Microscale electrodes have numerous useful applications. Amongst those applications, bio-electrochemical experiments play a prominent role. In order to comply with the vast variety of applications, the microelectrodes have to be adapted, e.g., in size, shape, strength, and structure. Moreover, the microelectrodes need to be adapted electrically, and have adequate chemical properties in order to be able to withstand the environments to which they have to be exposed to. Microelectrodes used for the interaction with biological matter also have to be bio-compatible.

Carbon materials have several attractive characteristics for microelectrodes, such as wide electrochemical potential window, good electrochemical activity, chemical stability, and simple functionalization of the surface. The most common carbon microfabrication techniques, like screen printing, produce planar two-dimensional structures. However, device sensitivity and biological signals measured with two-dimensional patterns are limited due to the two-dimensional nature of the electrodes. Hence, several three-dimensional microfabrication techniques have been explored in recent years. The so-called carbon MEMS technique is a simple and cost-effective method for carbon electrode fabrication, where a patterned polymer template treated at high temperature (typically around 1000 °C) in inert atmosphere (e.g. N_2 or Ar) is transformed into pyrolysed carbon electrodes. This process enables the fabrication of two-dimensional and, at least, quasi-three-dimensional electrodes with possibility for tailoring ad-hoc designs and unique sensitivities for specific applications. Due to this, pyrolysed carbon is becoming increasingly attractive for

numerous applications, such as novel sensors and scaffolds for tissue engineering. For relevant bio-electrochemical applications it is required that the electrodes are in the same order of magnitude as biological cells. However, high-throughput fabrication of electrically conductive, truly three-dimensional microstructures with a resolution down to a few microns still remains an unresolved challenge as evidenced by the following prior art disclosures.

The scientific article entitled “Fabrication of high-aspect ratio SU-8 micropillar arrays” published in Microelectronic Engineering 98 (2012) 483–487 by Amato et al. describes details of micromachining a high-aspect-ratio micro-pillar array in SU-8 using a UV-lithography process. The disclosed process includes low temperature baking steps to achieve an improved lateral resolution down to a few microns for high-aspect-ratio microstructures defined in SU-8 photoresist. The micropillar array may be used as a template for a corresponding pyrolysed carbon MEMS structure. However, the article is silent about how to produce a truly three-dimensional structure with hanging portions suspended at a distance above the substrate. Consequently, the article does not provide any hints about how to produce such a structure with hanging portions.

The scientific article entitled “Fabrication and application of a stacked carbon electrode set including a suspended mesh made of nanowires and a substrate-bound planar electrode toward an electrochemical/biosensor platform” published in Sensors and Actuators B 192 (2014) 796– 803 by Lim et al. relates to a biosensor for performing electrochemical measurements. The three-dimensional carbon electrode is obtained via pyrolysis of the corresponding 3D polymer structure consisting of a photoresist, such as SU-8. The three dimensional structure comprises a single level of microscale hanging portions suspended between block-shaped electrode portions. The complete structure including the hanging portions is fabricated using UV-lithography. The article emphasizes the difficulties in controlling the thickness of the suspended structure. As a solution to these difficulties, the article presents a complex process using a polymerization-stop-layer.

US 8,349,547 B1describes a method of defining a three-dimensional network of microstructures in photoresist with a subsequent pyrolysis step for forming a porous

conductive carbon structure. In this method, multiple coherent beams of UV-light are brought to interference within a photoresist layer, thereby achieving a periodically modulated exposure in a mask-less process. The method requires a complex optical setup, which is incompatible with common mask-based UV-lithography equipment.

The scientific article entitled “Fabrication and characterization of freestanding 3D carbon microstructures using multi-exposures and resist pyrolysis” published in J. Micromech. Microeng. 18 (2008) 035012 by Lee et al. discloses a fabrication process for multi-level SU-8 microstructures including suspended/hanging portions. The structures are then carbonized to obtain corresponding carbon microstructures with suspended/hanging portions. In one method, the structures were defined using two subsequent UV exposures, wherein a first, high-level exposure defines the supporting structure, and wherein a second, low-level exposure (“partial exposure”) defines the suspended/hanging portions. The article does not address the difficulties encountered when trying to control the thickness of the suspended microstructures by exposure doses and development kinetics. Accordingly, the article does not provide a viable solution to these difficulties.

The scientific article entitled “From MEMS to NEMS with carbon” published in Biosensors and Bioelectronics 20 (2005) 2181–2187 by Wang et al. relates to a further method of creating three-dimensional carbon structures with hanging portions suspended between neighboring vertical columns using UV-lithography patterning alone. However, the disclosed method relies on overexposure and different underdevelopment timing to obtain suspended resist networks. During pyrolysis, uniform shrinkage of the photoresist network results in a suspended carbon network with increased openings. The article emphasizes the difficulties in controlling the timing for underdevelopment because of its sensitivity to the photoresist thickness, exposure dose, and baking condition. As a solution to these difficulties, the article proposes doping the SU-8 photoresist with Fe_2O_3 nanoparticles. However, such an addition of Fe_2O_3nanoparticles severely limits the flexibility of the process for fabricating desired structures in a controlled manner. Furthermore, such addition of Fe_2O_3 may affect or even be prohibitive for use of the structure in certain bioelectrochemical applications.

The scientific article entitled “Fabrication of suspended carbon microstructures by e-beam writer and pyrolysis” published in Carbon 44 (2006) 2602–2607 by Malladi et al. relates to yet another method of creating three-dimensional carbon structures with hanging portions suspended between neighboring vertical columns. This method involves the use of e-beam lithography for defining hanging portions, which is an expensive direct write process that is inherently slow and, therefore, not well suited for production at high-volumes.

However, the prior art methods are either complex, require complex equipment or-produce structures that are much larger than what is needed for many microscale applications, such as analysing biological cells where electrodes with typical dimensions in the order of the size of cells are needed.

Therefore, there is an outspoken need for an economically viable, well controlled method of micro-fabricating such truly three-dimensional microelectrode structures with a resolution down to a few microns.

SUMMARY OF THE INVENTION

The present invention relates to microstructures fabricated by surface micromachining techniques on a substrate. The following terms are therefore to be understood with respect to the plane of a substrate surface on which the disclosed microelectrode structure is provided. The terms “horizontal” and “lateral” refers to directions parallel to the substrate surface. The term “vertical” refers to directions perpendicular to the substrate surface, i.e. perpendicular to the horizontal directions. The terms “up” or “upward” refer to directions having vertical components pointing away from the substrate. The terms “down” or “downward” refer to directions having vertical components pointing towards the substrate surface. Accordingly, the terms “top” and “bottom” as well as “above” and “below” denote spatial relationships with respect to the vertical directions, wherein “top” or “above” describes locations further away from the substrate surface than “bottom” or “below”.

Throughout the present application reference is made to the terms “truly three-dimensional” and “quasi three-dimensional”. The term truly three-dimensional struc-

tures as used herein is distinguished from the term “quasi-three-dimensional” structures in that the truly three-dimensional structures include so-called hanging structures that are supported by one or more vertical structures at a pre-determined height above a substrate (the height of the vertical supporting structure). At that height, the hanging structures freely project from the contour of the vertical supporting structure in a lateral direction. Typically, the hanging structures comprise bridges connecting two or more supporting structures at a predetermined height above a bottom end of the supporting structure.

When referring to “microns” it is to be understood that 1 micron = 1µm (one micrometer). Generally, microscale refers to dimensions typically measured in microns, such as between 1µm and up to 1000µm. In the present application the term microscale refers preferably to dimensions of about 1µm up to about 100µm.

According to one aspect, The present invention relates to a method of producing a three-dimensional microscale patterned resist template for a pyrolysed carbon microelectrode structure by means of UV-lithography, the method comprising the steps of: coating a planar substrate with a photoresist layer of an epoxy-based negative photoresist; soft baking the photoresist layer; performing a full depth exposure with UV light through a first mask, thereby defining a first latent image of a vertical support structure extending over the full depth of the photoresist layer; performing a partial depth exposure with UV light through a second mask, thereby defining a second latent image of a lateral structure in a top portion of the photoresist layer; wherein the full depth exposure and the partial depth exposure are aligned to ensure that the first and second latent images are connected to each other; post-exposure baking the photoresist layer, thereby creating a post-exposure bake latent image from the combined first and second latent images; and developing the microscale patterned resist template as a free-standing structure of cross-linked resist with lateral hanging structures that are supported by vertical support structures at a free height above the substrate; wherein soft baking is performed at temperatures not exceeding 70°C.

According to a more specific aspect, the epoxy-based negative photoresist is an SU-8 based photoresist. Accordingly, the present invention relates in one aspect to a

method of producing a three-dimensional microscale patterned resist template for a pyrolysed carbon microelectrode structure by means of UV-lithography, the method comprising the steps of: coating a planar substrate with a photoresist layer of an SU-8 based photoresist; soft baking the photoresist layer; performing a full depth exposure with UV light through a first mask, thereby defining a first latent image of a vertical support structure extending over the full depth of the photoresist layer; performing a partial depth exposure with UV light through a second mask, thereby defining a second latent image of a lateral structure in a top portion of the photoresist layer; wherein the full depth exposure and the partial depth exposure are aligned to ensure that the first and second latent images are connected to each other; post-exposure baking the photoresist layer, thereby creating a post-exposure bake latent image from the combined first and second latent images; and developing the microscale patterned resist template as a free-standing structure of cross-linked resist with lateral hanging structures that are supported by vertical support structures at a free height above the substrate; wherein soft baking is performed at temperatures not exceeding 70°C.

By this low temperature soft bake, a surprising control of the vertical thickness is achieved. Commonly in prior art procedures, and in agreement with the supplier's recommendations of how to process SU-8 resist, the soft bake is ramped or stepped up to 95°C and typically a major part of the resist drying is performed at 95°C. The inventors are aware of a previously disclosed process employing a soft bake procedure at temperatures significantly below the recommended soft bake temperature of 95°C, which has been published by the inventors themselves in the above-mentioned scientific article by Amato et al. However, the disclosed process is for optimizing the quality and for pushing the lateral resolution of full depth exposures for the fabrication of high-aspect ratio SU-8 micropillar arrays. The above-mentioned process is not at all for fabricating hanging structures.

Quite contrary to using a low temperature soft bake for the optimization of the exposure over the full depth of the photoresist layer, the present invention applies such a soft bake to control the depth of exposure in a vertical direction, thereby optimizing the vertical resolution in a partial depth exposure. The inventive insight of the present invention is based on the surprising observation that a soft bake performed at

temperatures not exceeding the 70°C in combination with a low exposure dose (the partial depth exposure dose) is surprisingly useful for performing a partial depth exposure of an SU-8 photoresist layer, and thereby producing lateral hanging structures with a reproducible vertical thickness in a surprisingly well controlled manner.

A substrate is coated with a layer of SU-8 negative tone photoresist. SU-8 is an epoxy-based negative photoresist which is widely used, and also commonly used in many MEMS applications. SU-8 derives its name from the presence of eight epoxy groups per monomer, which is a statistical average per moiety. The epoxy groups are responsible for the cross-linking to achieve the final structure. SU-8 resists are available in different formulations. Preferably, the SU-8 based resist is selected from the group of Microchem SU-8 series, SU-8 2000 series, and SU-8 3000 series photoresists or equivalent formulations. SU-8 negative photoresist formulations as specified above are available, for example, from MicroChem Corp., 1254 Chestnut Street, Newton, MA 02464, USA (www.microchem.com). These SU-8 resists are well-established in the art and are traceably well-defined by the above references in regard of all properties relevant to the present invention. Furthermore, the resists are described in respective datasheets (all of October 2011: SU-8 3000 Data Sheet; SU8_2-25; SU8_50-100; SU-82000DataSheet2000_5thru2015Ver4; SU-82000DataSheet2025thru2075Ver4; SU-82000DataSheet2100and2150Ver5) which are hereby incorporated by reference. Equivalent formulations of SU-8 photoepoxy negative resist are also available from e.g. Gersteltec Sarl., Générale Guisan 26, 1009, Pully, Switzerland (www.gersteltec.com), such as GM1040, GM1050, GM1060, GM1070, or GM1075, which are all described in respective datasheets (GM1040 datasheet of January 2005; GM1050 datasheet of October 2008; GM1060 datasheet of January 2005; GM1070 datasheet of January 2005; and GM1075 datasheet of June 2007), which are hereby incorporated by reference.

Typical SU-8 photoresist layer thicknesses used for high resolution fabrication of truly three-dimensional structures with hanging portions are between 20 µm and 100 µm.

After performing a soft bake as described above, the SU-8 resist layer is exposed with UV light. At least two separate exposures are performed: a full depth exposure

for defining vertical support structures extending vertically through the entire thickness of the resist layer and a partial depth exposure for defining lateral structures in a top portion of the same SU-8 layer. The lateral structures extend in a vertical direction from the top of the SU-8 layer in a downward direction with a vertical thickness that is only a fraction of the SU-8 resist layer thickness. The lateral structures are connected to the vertical support structures. Thereby, lateral “hanging” structures are patterned as a latent image into the resist by the partial depth exposure.

The partial depth exposure is typically performed at a very low exposure dose close to the threshold for generating any cross-linking in the SU-8 at all. For a specific structure to be produced a partial depth exposure dose may be determined by a routine test, such as an exposure dose matrix test varying the exposure dose of a corresponding layer of SU-8 on a corresponding substrate and selecting a value below the minimum exposure dose resulting in a full depth crosslinking exposure leaving a film on the surface after development. The correct partial depth exposure dose may be optimized with a test routine performed on the actual structure design to be produced. A non-limiting example of such a test routine is given below in the examples.

The latent images formed by the partial and full depth exposures are fixated by applying a post exposure bake that should not exceed the temperature of the soft bake step.

Finally, the pattern is developed by a state of the art developing procedure, wherein developing here includes developing the photoresist by the application of solvent to remove non-cross-linked resist, as well as any required rinsing, and drying steps. The method thus provides a polymer template that can be pyrolysed in order to produce a conductive carbon microscale electrode structure that is useful e.g. for microscale electrochemical experiments.

Further according to some embodiments, a soft baking time is adjusted to ensure a residual solvent concentration above 6%, preferably above 8%, preferably above 10%, or even above 15% by weight.

Commonly, during the soft baking, the photoresist is dried to a level where the residual solvent concentration stabilizes, i.e. the residual solvent concentration does not significantly decrease any more as a function of increased soft baking time. In the processes described in this application, however, the soft baking is preferably stopped well before the residual solvent concentration stabilizes as a function of baking time, so as to maintain an elevated residual solvent concentration as compared to an approximately stabilized drying level of the photoresist, where the photoresist would usually be considered as “fully dried”. A measure for the residual solvent concentration, and in particular whether or not the residual solvent concentration has stabilized, may e.g. be determined in a simple routine by weighing a wafer with the photoresist at different times throughout the soft baking procedure. The desired soft baking time for producing a particular structure may, of course, be determined in a simple off-line routine beforehand and independent of the actual production process, e.g. as part of the process calibration in the set-up phase of the production process for the particular structure. Alternatively or in addition thereto, the simple weighing procedure may be part of the metrics for controlling a given process.

The elevated residual solvent concentration aids in maintaining a certain mobility of the monomers in the photoresist, which facilitates cross-linking at lower temperatures. As a consequence both extrinsic stress between the patterned photoresist and the substrate and intrinsic stress within the patterned resist are reduced. Thereby an improved mechanical stability of the delicate truly three-dimensional structures is achieved. This is of particular importance when trying to achieve high resolution structures with critical dimensions of below 50µm, wherein the importance increases further as the critical dimensions are further reduced, to the point where keeping the SU-8 photoresist layer in a “wet state”, i.e. stopping the soft bake prior to reaching a more or less stable dry state of the SU-8 photoresist layer, becomes indispensable for achieving structures at the smallest scales, such as below 10µm.

In a contact pattern transfer process, an upper limit of the residual solvent concentration is given by the requirement that the photoresist layer may not adhere to the photo mask. In practice, the residual solvent concentration may be kept at a level

that just about avoids adherence to the photo mask during the photolithographic exposure.

Further according to some embodiments, the soft baking temperature is below 70°C, below 65°C, between 20°C and 60°C, preferably between 30°C and 55°C or about 50°C. Preferably, the soft bake temperature does not exceed 60°C, and more preferably the soft bake temperature is about 50°C, even though yet lower temperatures may be applicable for thinner layers, below the typical thicknesses between about 20µm and 100µm. Typical baking times associated with these soft bake temperatures are in the range of half hours or hours rather than a few minutes.

Further according to some embodiments, post-exposure baking is performed at a temperature equal to or below the soft baking temperature. Thereby a reduced extrinsic and intrinsic stress in the polymer template structure is achieved.

Further according to some embodiments, vertical critical dimensions of the microscale patterned resist template produced by the method are less or equal to 40 microns, less or equal to 30 microns, less or equal to 20 microns, or even less or equal to 10 microns, wherein the vertical critical dimensions are one or more of the vertical free height between the substrate and the lateral hanging structures, and the vertical thickness of the lateral hanging structures. These dimensions are particularly useful for bio-electrochemical applications, where the microelectrodes also have to be suited for performing the function of a cell/tissue growth scaffold, which puts strict constraints on the useful scale. In particular, a bio-electrochemical sensor with a truly three-dimensional microelectrode is more sensitive for measurements on cells if the dimensions are small, preferably in the same order of magnitude as the cells, i.e. less or equal to 10µm.

Further according to some embodiments, lateral critical dimensions of the microscale patterned resist template are less or equal to 40 microns, less or equal to 30 microns, less or equal to 20 microns, or even less or equal to 10 microns, wherein the lateral critical dimensions are one or more of a lateral transverse dimension of the support structure, a lateral distance between adjacent support structures, and a lateral width of the lateral hanging structure. As mentioned above, these dimensions

are particularly useful for bio-electrochemical applications, where the microelectrodes also have to be suited for performing the function of a cell/tissue growth scaffold, which puts strict constraints on the useful scale.

Further according to some embodiments, a plurality of vertical support structures of the microscale patterned resist template are arranged in a periodic manner in at least one lateral direction. Thereby the microelectrode template is e.g. useful for producing electrodes that are particularly advantageous for also acting as a scaffold for biological cells/microorganisms.

Further according to some embodiments, the hanging structures are formed so as to connect neighbouring support structures. The hanging structures connecting neighbouring support structures may e.g. be formed as beams or bridges, as perforated lateral webs, filigree, membranes, or the like.

Further according to some embodiments, the method further comprises, after performing the partial depth exposure of the photoresist layer and after post-exposure baking: applying one or more further photoresist layers; soft baking each of the further photoresist layers, and performing on each of the further photoresist layers a partial depth exposure with UV light through respective further masks followed by post-exposure baking, thereby defining in each further photoresist layer in a top portion thereof a respective latent image of a further lateral structure. Thereby a microscale patterned resist template is obtained having multiple levels of lateral hanging structures that are supported by vertical supporting structures at a free height above (the top of) the supporting level; the total height of each level is determined by the thickness of the photoresist layer of that level.

Further according to some embodiments, a full depth exposure is performed for each further photoresist layer. By performing a full depth exposure for each layer, a good control of the achievable resolution is achieved for each layer. This may be of particular significance for layers with a single layer thickness of several hundreds of microns. Furthermore, the design of subsequent levels can be chosen with a large degree of architectural freedom only subject to the constraint that subsequent levels

need to be mechanically connected in order to provide appropriate support of upper levels by lower levels.

Further according to some embodiments, a single full depth exposure is performed for a plurality of subsequent/vertically adjacent /neighbouring layers at the same time/in one go. Combining the full depth exposures for a plurality of photoresist layers has the advantage that no alignment issues of vertical support elements of one level with respect to other levels arise, and that one or more exposure steps are skipped. An upper limit for this shortcut is determined by the total thickness to be exposed by such a combined full depth exposure covering multiple layers.

Further according to some embodiments, full depth exposures and partial depth exposures are performed at the same UV wavelength, such as at the i-line at 365nm. By using the same wavelength for all exposures, a more simple process flow is achieved, since the exposures can be performed on a single mask aligner and no interfering with the exposure optics by changing the exposure wavelength is required. Preferably, a deep UV line above 360nm is chosen in agreement with the optical properties of common SU-8 formulations, such as those recited above. Preferably, the exposure is performed at the i-line, at 365 nm. Thereby, a high resolution precise imaging is achieved, which is of particular relevance for the quality of the pattern transfer for full depth exposure.

Further according to some embodiments, full depth exposures are performed at a first UV wavelength, such as the i-line at 365nm, and the partial depth exposures are performed at a second UV wavelength different from the first UV wavelength, wherein the absorption of UV light by the SU-8 based resist is higher at the second UV wavelength as compared to the first UV wavelength. Performing exposures at separate wavelengths allows for adapting the optics to the type of the exposure for pattern transfer, and accordingly to separately optimizing the optics of the full depth exposure pattern transfer and of the partial depth exposure pattern transfer. Advantageously, a deep UV line above 360nm is chosen for the full depth exposure in agreement with the optical properties of common SU-8 formulations, such as those recited above. Preferably, the full depth exposure is performed at the i-line, at 365 nm. Thereby a high resolution precise imaging is achieved for the pattern transfer in

the full depth exposure. In contrast thereto, a lower wavelength, preferably a wave length below 360 nm is chosen for the partial depth exposure. At such shorter wavelengths, the SU-8 resist layer is more absorptive than at the longer wavelengths, thus allowing for a better control of the penetration depth for the partial depth exposure.

According to a further aspect, a method of producing a three-dimensional microscale patterned resist template for a pyrolysed carbon microelectrode structure by means of UV-lithography is provided, the method comprising:

- coating a planar substrate with a first photoresist layer of a first epoxy-based negative photoresist;
- soft baking the first photoresist layer;
- performing a full depth exposure with UV light through a first mask, thereby defining a first latent image of a vertical support structure extending over the full depth of the first photoresist layer;
- post-exposure baking the first photoresist layer;
- coating the first photoresist layer with a second photoresist layer of a second epoxy-based negative photoresist different from the first epoxy-based negative photoresist, thereby forming a layer stack comprising the first and second photoresist layers;
- soft baking the second photoresist layer;
- performing a partial depth exposure with UV light through a second mask, thereby defining a second latent image of a lateral structure in the second photoresist layer; wherein the full depth exposure and the partial depth exposure are arranged to ensure that the first and second latent images are connected to each other;
- wherein the partial depth exposure is performed using UV-light having a different spectral intensity distribution than the UV-light for the full depth exposure; wherein the second epoxy-based negative photoresist is more sensitive to the UV-light of the partial depth exposure than the first epoxy-based negative photoresist;
- post-exposure baking the second photoresist layer; and

- developing the microscale patterned resist template as a free-standing structure of cross-linked resist with lateral hanging structures that are supported by vertical support structures at a free height above the substrate;
- wherein soft baking is performed at temperatures not exceeding 70 °C.

Thereby an enhanced control of the vertical thickness of the hanging structures may be achieved as compared to the above-mentioned embodiments. This is achieved by using a first photoresist layer with a first spectral sensitivity for defining a latent image of vertical support structures, and a second photoresist layer with a second spectral sensitivity on top of the first photoresist layer for defining the hanging structures. The first and second photoresist layers have different spectral sensitivities, and the respective full and partial depth exposures are performed at respective wavelengths matching these spectral sensitivities. The full depth exposure is performed at a first wavelength, such as at wavelengths corresponding to the i-line at 365 nm, wherein the spectral sensitivity of the first photoresist layer of the first epoxy-based negative resist is adapted for efficient cross-linking at the first wavelength(s). The partial depth exposure, on the other hand, is performed at a second wavelength different from the first wavelength, such as at wavelengths of about 405 nm, wherein the spectral sensitivity of the second photoresist layer of the second epoxy-based negative resist is adapted for efficient cross-linking at the second wavelength(s). However, the spectral sensitivities of the two different epoxy-based resists are chosen or configured such that the first photoresist layer is much less sensitive to the UV-light of the partial depth exposure than the second photoresist layer. Therefore the latent image of the lateral/hanging structures may effectively be confined to the top layer, i.e. to the second photoresist layer, and the thickness control is mainly a matter of controlling the layer thickness of the second photoresist layer. It may be noted that this further enhanced thickness control is achieved at the expense of additional process steps, including the need of handling multiple resists and exposure at different wavelengths. It should further be noted that in agreement with the above-mentioned embodiments of the invention, both the thickness control and the stability of the process are apparently improved by performing soft baking at temperatures not exceeding 70 °C.

Further according to some embodiments of the method, the first epoxy-based negative photoresist is adapted for cross-linking with a maximum sensitivity to UV-light in a first range at wavelengths below 380nm, such as between 350nm and 380nm. This range is compatible with a spectral region commonly used for UV-lithography, namely the spectral region comprising the so-called i-line at about 365 nm.

Further according to some embodiments of the method, the second epoxy-based negative photoresist is adapted for cross-linking with a maximum sensitivity to UV-light in a second range at wavelengths above 380nm, such as between 380nm and 420nm. By choosing the second spectral region for the spectral sensitivity such that cross-linking already occurs at longer wavelengths for the second photoresist layer as compared to the first layer, the cross-linking response of the first photoresist layer to the partial depth exposure is effectively suppressed, thereby (further) enhancing the reliability of layer thickness control.

Further according to some embodiments of the method, the first epoxy-based negative photoresist is an SU-8 based photoresist. Further according to some embodiments of the method, the soft baking temperature is between 20 °C and 60 °C, preferably between 30 °C and 55 °C or about 50 °C. Yet further according to some embodiments of the method, post-exposure baking is performed at a temperature equal to or below the soft baking temperature. By the additional features of these embodiments at least the same additional advantages are achieved as already mentioned above for the corresponding features. Furthermore, the skilled person will understand that in analogy to what was described above, the relevant steps prior to developing may be repeated in order to create truly three-dimensional structures with multiple levels. Also, periodically arranged support structures and truly three-dimensional structures where the hanging structures connect neighbouring support structures may be produced using this embodiment, thereby achieving at least the above-mentioned advantages for the corresponding configurations.

Further according to one aspect, a method of fabricating a pyrolysed carbon microelectrode structure on a substrate is provided, the method comprising: providing a microscale patterned resist template by a method according to any of the above-mentioned methods; and pyrolysing the microscale patterned resist template to ob-

tain a pyrolysed carbon microelectrode structure with one or more laterally extending hanging portions suspended by one or more vertical support structures at a height above the substrate.

The carbon microelectrodes thus fabricated by pyrolysing a truly three-dimensional template microstructure made from SU-8 have a large variety of useful applications. For example, they can be used as electrodes for bio-electrochemical applications, sensors, and tissue engineering. The high-resolution and truly three-dimensional conductive carbon structures with critical dimensions of a few microns and up to a few tens of microns, such as in the range of 1-40µm, are particularly well-suited for bio-electrochemical cell-analysis applications where the microscale carbon electrodes are used as scaffolds for growing cell-cultures of cells to be analysed. Examples for such cell cultures may include human neural stem cell cultures, which may be differentiated and analyzed on the truly three-dimensional carbon microstructures.

According to a further aspect, a truly three-dimensional microelectrode structure made of pyrolysed carbon is fabricated as a working electrode for use in an electrochemical cell. The truly three-dimensional carbon microelectrode structure has vertical support columns arranged on a substrate. The support columns are connected to each other by a network of lateral hanging beams at one or more levels above the substrate. When dimensioned with critical dimensions below 50µm, an electrochemical cell-based sensor device is obtained that is useful for performing bio-electrochemical cell analysis measurements on living cells, and in particular on cell cultures, wherein the truly three-dimensional structure of the carbon microelectrode acts as a scaffold for differentiation and/or growth of cells in direct contact or in in close proximity of the working electrode.

According to yet a further aspect, a microfluidic electrochemical cell according to some embodiments comprises at least a working electrode, a counter electrode, and a reference electrode, wherein the working electrode comprises a truly three-dimensional microelectrode structure of pyrolysed carbon as described herein. Most preferably, the truly three-dimensional microelectrode of pyrolysed carbon is fabricated according to any of the methods described herein.

As mentioned above, the truly three-dimensional carbon microelectrodes for bio-electrochemical applications according to some embodiments of the present invention have at least one level of lateral hanging structures supported by vertical support structures. Preferably at least two levels or more of lateral hanging structures are provided.

Multi-layered truly three-dimensional porous and conductive carbon scaffolds can be applied for several applications, including physiological studies of cells, toxicological evaluations, and neurophysiological investigations.. In such applications, the multi-layered scaffold facilitates energy conversion by maximizing the active surface area and by minimizing the diffusion lengths. Truly three-dimensional scaffolds that are conductive and integrated in an electrode provide optimal properties for cell culturing and can be used for in situ three-dimensional electrochemical analysis of cell growth and cell response to external stimuli such as environmental pollutants.

BRIEF DESCRIPTION OF THE DRAWINGS

Preferred embodiments of the invention will be described in more detail in connection with the appended drawings, which show in

Fig. 1 a schematic of a process flow according to a first embodiment for providing a pyrolysed single level structure;

Fig. 2 a schematic of a process flow according to a second embodiment for providing a pyrolysed multi-level structure;

Fig. 3 a scanning electron micrograph of a pyrolysed single level structure obtained by process according to the first embodiment (scale bar 10µm);

Fig. 4 a scanning electron micrograph of a pyrolysed single level structure obtained by a process according to the first embodiment (scale bar 20µm);

Fig. 5 a scanning electron micrograph of a pyrolysed multi-level structure obtained by a process according to the third embodiment (scale bar 20µm);

Figs. 6a-d a series of scanning electron micrographs illustrating the optimization of the vertical crosslinking depth with respect to UV exposure dose during partial depth exposure: (A) underexposure with 21mJ/cm^2 at 365nm (scal bar 100µm); (B) correct exposure with 28mJ/cm^2 at 365nm scale bar 100µm); (C) overexposure with 35mJ/cm^2 at 365nm scale bar 20µm); and (D) overexposure with 42mJ/cm^2 at 365nm (scale bar 100µm);

Figs. 7a-d a schematic of a process flow according to a further embodiment for providing a pyrolysed structure; and in

Figs 8a-k a schematic of a process flow for providing a carbon electrode in a layout for use in a microfluidic electrochemical cell.

DETAILED DESCRIPTION OF PREFERRED EMBODIMENTS

Fig.1 shows schematically a process flow according to a first embodiment for providing a pyrolysed single level carbon microelectrode structure with hanging lateral elements supported by vertical support elements. An SU-8 negative tone photoresist layer 2 is applied by spin coating to a substrate 99 and prepared for subsequent processing by soft baking at a temperature not exceeding 70°C. The soft baking is for removing excess solvent from the photoresist layer. Preferably, the soft baking time is adjusted to maintain an elevated residual solvent concentration well above that of a fully dried state as discussed above. The desired soft baking time for producing a particular structure may be determined in a simple off-line routine beforehand as also described above.

After the soft baking step, a full depth exposure with UV light through a first mask M1 is performed using a full depth exposure dose D1. Thereby, a first latent image 4 of a vertical support structure extending over the full depth of the photoresist layer 2 is defined as seen in Fig.1a.

A partial depth exposure of the photoresist layer 2 is now performed with UV light through a second mask M2 at a partial depth exposure dose D2 that is significantly lower than the full depth exposure dose. The partial depth exposure dose is adapted so as to define a second latent image of lateral structures 5 in a top portion of the photoresist layer 2, but the partial depth exposure dose D2 is not sufficient to bring about a crosslinking of the resist that would allow for defining a latent image over the full depth of the photoresist layer. The latent images of the full depth exposure and of the partial depth exposure are aligned with respect to each other to provide connections between the latent images of the vertical support elements 4 and of the lateral elements 5 as seen in Fig.1b. The photoresist layer 2 is then subjected to a post exposure bake to fixate the latent images and obtain a combined post-exposure bake latent image with full depth vertical elements and partial depth lateral elements at the top of the photoresist layer 2.

The structure is then developed by applying an appropriate developer removing the portions of the photoresist layer that are insufficiently crosslinked in a solvent, followed by rinsing, and drying. Preferably at this stage, an additional flood exposure of the freestanding photoresist structure with a high flood exposure dose is performed followed by a hard bake. This additional flood exposure and hard-bake is for enhancing cross-linking and thereby enhancing the mechanical stability of the freestanding three-dimensional polymer structure. The freestanding microscale polymer template with lateral elements 7 supported by vertical support elements 6 is seen in Fig.1c.

The template is then pyrolysed using a known carbon MEMS carbonization process for converting SU-8 polymer templates into conductive carbon structures. The pyrolysis step involves heating the template in an inert atmosphere to high temperatures, e.g. in Ar, H_2 or N_2 or mixtures of N_2 and H_2 to temperatures of about 900-1100°C. Thereby, the final microscale electrode of conductive carbon is obtained as a single level structure as seen in Fig.1d.

While in the process flow diagram of Fig.1 the full depth exposure (Fig.1a) is shown to occur prior to the partial depth exposure (Fig.1b), it should be noted that the sequence of these exposures is interchangeable.

In order to build a multiple level structure, instead of proceeding to developing, the steps illustrated in Figs.1a and b may be repeated as often as necessary, wherein each set of lateral hanging structures patterned by partial depth exposure of further resist layers define the further levels. After UV-exposure and prior to applying any subsequent layer, a post-exposure bake is performed on each of the photo-resist layers in order to fixate the latent image. This is in particular necessary for fixating the latent images created by partial depth exposures.

Fig.2 illustrates such a multi-level process for a two layer-structure. The steps of Fig.2a and Fig.2b are performed in the same manner as described above with respect to Fig.1a and Fig.1b, respectively. Furthermore, the first photoresist layer 2 is subjected to a post exposure bake to fixate the latent images and obtain a combined post-exposure bake latent image of a first level structure with vertical elements extending over the full depth of the first photoresist layer 2, and partial depth lateral elements at the top of the first photoresist layer 2.

As seen in Fig.2c, a second layer 12 of SU-8 photoresist is spun on top of the first photoresist layer 2 and prepared for exposure by applying a soft bake at a temperature not exceeding 70°C as explained above.

After soft baking of the second photoresist layer 12, a full depth exposure with UV light through the first mask M1 is performed using the full depth exposure dose D1. Thereby, a first latent image 14 of a vertical support structure extending over the full depth of the second photoresist layer 12 is defined. A partial depth exposure of the second photoresist layer 12 is now performed with UV light through the second mask M2 at the partial depth exposure dose D2 that is significantly lower than the full depth exposure dose D1. The partial depth exposure dose D2 is adapted so as to define a second latent image of lateral structures 15 in a top portion of the photoresist layer 12, but the partial depth exposure dose D2 is not sufficient to bring about a crosslinking of the resist that would allow for defining a latent image over the full depth of the second photoresist layer 12. The latent images 14, 15 of the full depth exposure and of the partial depth exposure are aligned with respect to each other to provide connections between the latent images of the vertical support elements 14

and of the lateral elements 15. Furthermore, the latent image of the vertical support elements 14 in the second photoresist layer 12 needs to be aligned with the post exposure bake latent image of the first photoresist layer 2 so as to ensure proper mechanical support of the second level structures. This can be seen in Fig.2c.

The assembly of the first and second photoresist layers with latent images is then again subjected to a post exposure bake to fixate the latent images 14, 15 and obtain a combined post-exposure bake latent image with full depth vertical elements and partial depth lateral elements at the top of the first and second photoresist layers 2, 12, respectively. The procedure may be repeated as often as necessary to build a multi-level structure layer by layer, wherein the latent images in all levels need to be aligned appropriately to provide proper mechanical connection between the levels. This is a matter to be taken into account in the architectural design of the structure to be built.

After the post exposure bake, the structure is developed by applying an appropriate developer removing the portions of the photoresist layer that are insufficiently cross-linked in a solvent, followed by rinsing, and drying. Preferably at this stage, an additional flood exposure of the freestanding photoresist structure with a flood exposure dose is performed followed by a hard bake. This additional flood exposure and hard-bake is for enhancing cross-linking and thereby enhancing the mechanical stability of the freestanding three-dimensional polymer structure. The freestanding microscale polymer template with lateral elements 7, 17 supported by vertical support elements 6, 16 is seen in Fig.2d.

The template is then pyrolysed using a known carbon MEMS pyrolysis process for converting SU-8 polymer templates into conductive carbon structures as described above. Thereby, the final microscale electrode of conductive carbon is obtained as a multi- level structure as seen in Fig.2e.

Again, as for Fig.1, it is also possible in the process flow of Fig.2 to interchange the sequence of the full depth exposures in Figs.2a and 2c with the partial depth exposures in Figs.2b and 2c. This may further be exploited in a variation of the process flow for creating a multi-level structure, by omitting the initial full depth exposure step

of the first layer shown in Fig.2a, and instead perform the full depth exposure for both the first and the second layer of photoresist (or of a plurality of photoresist layers) at the same time in one go. This has the advantage that no alignment issues of vertical support elements of one level with respect to other levels arise, and that at least one exposure step is omitted. An upper limit for this shortcut is determined by the total thickness to be exposed by such a combined full depth exposure covering multiple layers.

EXAMPLES

In the following, examples of process parameters are given. Examples are given for the fabrication of single level structures (examples 1 and 2 with reference to figs.3 and 4), for the fabrication of a multi-level structure (example 3 with reference to fig.5), and for the optimization of the partial depth exposure dose (example 4 with reference to fig.6a-d). All examples have been produced on <100> oriented silicon wafers using the negative tone epoxy photoresist SU-8 2075 available from Micro-Chem Corp. (USA). Pyrolysis of the structures given in examples 1-3 was performed in an inert atmosphere of nitrogen gas (N_2) at 900°C. Spin coating was performed on an RCD8 T spin coater from Süss Micro Tec. For the exposure steps, an MA6/BA6 mask aligner (Karl-Süss, France) was used. The mask aligner was equipped with a mercury lamp. A so-called SU-8 filter was used. The SU-8 filter has no transmission in the 250-330nm range, and close to full transmission in the 400-500nm range. In the 350-370nm range, the transmission is approximately 0.5.

Approximately 10ml of SU-8 2075 were manually dispensed in the center of a Si-substrate and spin-coating was performed using a two-step spin process. A spread cycle of 30s at 700rpm with 100rpm s^{-1} acceleration was applied to ensure that the deposited SU-8 was spread across the entire wafer. Subsequently, the wafer was spun at 2100rpm for 60sec with 100rpm s^{-1} acceleration yielding a 76µm thick film. The edge bead was removed by dispensing propylene glycol methyl ether acetate (PGMEA) at the edge of the wafer rotating at 300 rpm.

To determine the effect of the baking steps on the fabrication of the structures, temperature, ramping rate, and baking time of the soft bake as well as of the post exposure bake were varied in a series of experiments. A programmable hotplate (Harry Gestigkeit GmbH, Germany) for parallel processing of four samples was used for all the baking steps. The wafers were placed on a cold hotplate and ramped to the baking temperature. At the end of the baking time, the wafers were allowed to cool on the hotplate to room temperature by natural cooling over a period of 1.5– 2 h. An optimized soft baking temperature of 50 °C for 6hr with a ramp of 2 °C/min was used before the exposure. The long, low temperature baking step has surprisingly proven to provide a very good control of the vertical thickness of lateral hanging structures created by partial depth exposure patterning as described above.

After soft baking, the SU-8 layer is patterned by full depth UV exposure in soft contact mode through a first mask for defining a support structure array. The first mask includes hole-arrays with various dimensions (10µm – 100µm) and with a varying pitch (10µm – 500µm). The full depth exposure dose (D1) was optimized to achieve the highest lateral resolution. Exposure doses D1 from 100 to 210 mJ/cm^2 were tested and an optimized full depth exposure dose D1=147 mJ/cm^2 was selected for the full depth exposure. A second UV exposure for a partial depth exposure through a second mask is performed with a partial depth exposure dose (D2) at short UV wavelengths to obtain the overhanging layer. The second mask contains structures that connect the pillars in lateral directions thus forming a network of lateral beams connecting the vertical support structures at their top end to each other (dimension range from 5 µm – 500µm). The partial depth exposure dose, D2, was varied in the range from 21 – 42 mJ/cm^2 so as to crosslink only the top layer of SU-8. An optimized partial depth exposure dose of D2=28 mJ/cm^2 was selected for the partial depth exposure. For the post expose bake, an optimized baking temperature of 50 °C for 8h with a ramp of 2 °C/min followed by cooling down to room temperature was used before the development. The development in propylene glycol methyl ether acetate (PGMEA) was performed in two steps of 10 min. First a rough development to remove most of the unexposed SU-8 was done in a first developer bath, after which each wafer was transferred to a second developer bath. Development was terminated by an isopropanol rinse for 30 s and subsequently the wafers were dried by evaporation of the rinse solvent, either in air or using supercritical drying with CO_2. For the supercritical drying, the specimens were placed in an isopropanol

bath directly after development and then transferred to an Autosamdri-810 CO_2 supercritical point dryer (Tousimis, USA). In an optional procedure after drying, an additional two-step flood exposure with the total D = 500 mJ/cm^2 and a hard-bake at 90°C for 15 h on the hot plate were tested. This optional procedure is useful for increasing the crosslinking of the polymer, thereby enhancing temporal-stability of the SU-8 template structures.

The polymer template is pyrolysed in an inert atmosphere (N_2) at 900 °C for 1h with a ramp of 2 °C/min to produce carbon microstructures in an ATV furnace (Germany). After pyrolysis, microscale conductive carbon structures were reliably obtained at a high resolution.

Example 1

Fig. 3 shows a scanning electron micrograph of a pyrolysed single level structure obtained by a process according to the first embodiment (scale bar 10µm), wherein an array of support columns, created by full depth exposure of a periodic hole pattern, carries a single level of a lateral network of interconnecting beams, which have been created by partial depth exposure of a regular square lattice pattern, wherein intersection points of the square lattice are aligned with the support columns. The vertical support columns are placed with a lateral periodicity of 50 µm. The total height of the single level carbon structure is 36 µm. The openings in the hanging layer have a lateral width of about 39 µm as seen in the directions parallel to the beams. The following recipe summarizes the process flow and parameters used for the fabrication of the structure shown in Fig.3:

- Clean substrate in H_2SO_4/H_2O_2, prebake for 2 h at 200 °C
- Spin-coat SU-8 2075 to a thickness of 76 µm in two steps:
  (1) 30 sec at 700 rpm with 100 rpm/sec acceleration
  (2) 60 sec at 2100 rpm with 100 rpm/sec acceleration
- Soft bake at 50 °C for 6 h with a ramp of 2 °C/min
- First UV exposure (full depth): D1=147 mJ/cm^2, mask 1, 365 nm light
- Second UV exposure (partial depth): D2=28 mJ/cm^2, mask 2, 365 nm light
- Post-exposure bake at 50 °C for 8 h with a ramp of 2 °C/min
- Develop: 10mins (first bath) + 10mins (second bath) in PGMEA
- Flood Exposure : 500 mJ/cm^2, 365 nm light

- Hard bake: 90 °C for 15 h with a ramp of 2 °C/min
- Pyrolysis: 900 °C for 1 h with a ramp of 2 °C/min in a nitrogen atmosphere (N_2)

Example 2

Fig. 4 shows a scanning electron micrograph of another pyrolysed carbon single level structure obtained by a process according to the first embodiment (scale bar 20µm). The same process flow with the same process parameters as in example 1 has also been used in example 2 to fabricate the structure shown in Fig.4. Note the mesh pattern with critical lateral and vertical dimensions of about 4 µm, which has been created by the partial depth exposure. The lateral hanging pattern is carried by a regular array of support columns created by the full depth exposure. The support columns are placed in a square lattice arrangement with a lateral periodicity of 50 µm, and are connected with each other at their top by lateral beams with a lateral width of about 18 µm forming the square edge portions of the filigree mesh. The total height of the single level carbon structure is 36 µm.

Example 3

Fig. 5 shows a scanning electron micrograph of a pyrolysed multi-level structure obtained by a process according to a further embodiment (scale bar: 20 µm). First and second levels of a square lattice structure corresponding to that of example 1 have been created on top of each other. The vertical support columns are placed with a lateral periodicity of 50 µm. The lateral beams have a critical dimension in a lateral direction transverse of the beams of about 18 µm. The structure has been fabricated using a multi-level process flow as summarized in the following recipe:

- Clean substrate in H_2SO_4/H_2O_2, prebake for 2 h at 200 °C
- Spin-coat SU-8 2075 to a thickness of 76 µm in two steps:
  (1) 30 sec at 700 rpm with 100 rpm/sec acceleration
  (2) 60 sec at 2100 rpm with 100 rpm/sec acceleration
- Soft bake at 50 °C for 6 h with a ramp of 2 °C/min
- UV exposure (partial depth): D2=28 mJ/cm^2, mask 2, 365 nm light
- Post-exposure bake: 50 °C for 6 h with a ramp of 2 °C/min

- Spin-coat SU-8 2075 to a thickness of 76 µm in two steps:
  (1) 30 sec at 700 rpm with 100 rpm/sec acceleration
  (2) 60 sec at 2100 rpm with 100 rpm/sec acceleration
- Soft bake at 50 °C for 8h with a ramp of 2 °C/min
- UV exposure (partial depth): D2=28 mJ/cm^2, mask 2, 365 nm light
- UV exposure (full depth): D1=147 mJ/cm^2, mask 1, 365 nm light
- Post-exposure bake at 50 °C for 8 h with a ramp of 2 °C/min
- Develop: 15 mins (first bath) + 15 mins (second bath) in PGMEA
- Flood Exposure : 500 mJ/cm^2, 365 nm light
- Hard bake: 90 °C for 15 h with a ramp of 2 °C/min
- Pyrolysis: 900 °C for 1 h with a ramp of 2 °C/min in a nitrogen atmosphere (N_2)

Example 4

Figs.6a-d show a series of scanning electron micrographs of single level polymer templates illustrating the optimization of the vertical cross-linking depth with respect to UV exposure dose during partial depth exposure. Essentially the same process flow and parameters as for examples 1 and 2 has been used, only omitting the pyrolysis step and varying the partial depth exposure dose as follows:

- Fig.6a: underexposure (21 mJ/cm^2 at 365 nm);
- Fig.6b: correct exposure (28 mJ/cm^2 at 365 nm);
- Fig.6c: overexposure (35 mJ/cm^2 at 365 nm); and
- Fig.6d: overexposure (42 mJ/cm^2 at 365 nm).

Example 5

Example 5 provides a proces flow for fabricating a three-dimensional microscale patterned resist template for use in a pyrolysed carbon microelectrode structure using UV-lithography, The process includes using a layer stack comprising two different photoresissts on top of each other, which are exposed at respective wavelengths. By this porcess, a reliable vertical thickness control of the lateral hanging structures is achieved:

- Clean Substrate in H_2SO_4/H_2O_2, Prebake for 2 h at 200 ^oC
- Spin-coat SU-8 2075 to a thickness of 76 µm in two steps:

- 30 sec at 700 rpm with 100 rpm/sec acceleration
- 60 sec at 2100 rpm with 100 rpm/sec acceleration
- Soft bake at 50 °C for 5 h with a ramp of 2 °C/min
- First UV exposure (full depth): D1=147 mJ/cm2, mask 1, 365 nm light
- Post-exposure bake at 50 °C for 5 h with a ramp of 2 °C/min
- Spin-coat mr-DWL 40 at 60 sec at 2100 rpm with 500 rpm/sec acceleration to get a thickness of 17 µm
- Soft bake at 50 °C for 1 h with a ramp of 2 °C/min
- Second UV exposure: D2=25 mJ/cm2, mask 2, 405 nm light
- Post-exposure bake at 50 °C for 1 h with a ramp of 2 °C/min
- Develop: 10mins (first bath) + 10mins (second bath) in PGMEA and rinse in ISO
- Flood Exposure : 500 mJ/cm2, 365 nm light
- Hard bake: 90 °C for 15 h with a ramp of 2 °C/min
- Pyrolysis: 900 °C for 1 h with a ramp of 2 °C/min in a nitrogen atmosphere (N2)

The process flow is schematically summarized in Figs. 7a-d, wherein Fig.7a shows the definition of a latent image of a supporting structure by means of UV-lithography in a first photoresist layer cross-linking upon exposure with UV-light at a wavelength of 365 nm (“full depth exposure”); Fig.7b shows the definition of a latent image of a hanging structure by means of UV-lithography in a second photoresist layer cross-linking upon exposure with UV-light at a wavelength of 405 nm (“partial depth exposure”); Fig.7c shows the developed structure with all non-cross linked photoresist removed; and Fig.7d shows the pyrolysed carbon structure. The difference in wavelength helps in limiting the second exposure to the top layer in order to achieve that crosslinking by the second exposure is essentially limited to the second photoresist layer. By repeating the steps summarized by Figs.7a and 7b, followed by a final development for the removal of non-cross-linked material will result in a truly three-dimensional polymer template with multiple levels. The template can then be pyrolyzed to obtain a truly three-dimensional carbon microelectrode with multiple levels.

Example 6

Example 6 provides a further proces flow including fabricating a three-dimensional microscale patterned resist template for use in a pyrolysed carbon microelectrode structure using UV-lithography. The process further includes steps leading to producing a microfluidic electrochemical cell comprising at least a working electrode, a counter electrode, and a reference electrode, or a working electrode and a counter electrode, wherein the working electrode comprises a truly three-dimensional microelectrode structure of pyrolysed carbon:

- Thermal oxidation of SiO_2 on Si
- Clean substrate in H_2SO_4/H_2O_2, prebake for 2 h at 200 °C
- Spin-coat SU-8 2035 at 120 sec at 5000 rpm with 500 rpm/sec acceleration to get a thickness of 15 µm
- Soft bake at 50 °C for 15 min with a ramp of 2 °C/min
- First UV exposure (full depth): D1=147 mJ/cm2, mask 1, 365 nm light
- Post-exposure bake at 50 °C for 1 h with a ramp of 2 °C/min
- Spin-coat SU-8 2075 to a thickness of 76 µm in two steps:
- 30 sec at 700 rpm with 100 rpm/sec acceleration
- 60 sec at 2100 rpm with 100 rpm/sec acceleration
- Soft bake at 50 °C for 6 h with a ramp of 2 °C/min
- First UV exposure (full depth): D1=147 mJ/cm2, mask 2, 365 nm light
- Second UV exposure (partial depth): D2=28 mJ/cm2, mask 3, 365 nm light
- Post-exposure bake at 50 °C for 8 h with a ramp of 2 °C/min
- Develop: 10mins (first bath) + 10mins (second bath) in PGMEA
- Flood Exposure : 500 mJ/cm2, 365 nm light
- Hard bake: 90 °C for 15 h with a ramp of 2 °C/min
- Pyrolysis: 900 °C for 1 h with a ramp of 2 °C/min in a nitrogen atmosphere (N2)
- E-beam deposition of Au reference and contact pads through a shadow mask
- Spin-coat SU-8 2005 at 60 sec at 2500rpm with 500 rpm/sec acceleration to get a thickness of 5 µm
- Soft bake at 50 °C for 15 min with a ramp of 2 °C/min

- First UV exposure (full depth): D1=147 mJ/cm2, mask 4, 365 nm light (to define the working area, we add a passivation layer, figure k)
- Post-exposure bake at 50 °C for 1 h with a ramp of 2 °C/min
- Develop: 2mins (first bath) + 2mins (second bath) in PGMEA

The above process flow for producing a carbon electrode for use in a microfluidic electrochemical cell is schematically illustrated in Figs 8a-k. The electrodes on the microelectrode chips shown in Fig.8k include, from left to right, a C-shaped counter-electrode (left), a working-electrode (centre), and a reference electrode (right).

By way of analogy the steps of producing a photoresist template, here shown in Figs.8a-h, may be replaced by any of the methods of producing a three-dimensional microscale patterned resist template for a pyrolysed carbon microelectrode structure by means of UV-lithography, as discussed herein.

CLAIMS

1. Method of producing a three-dimensional microscale patterned resist template for a pyrolysed carbon microelectrode structure by means of UV-lithography, the method comprising:
   - Coating a planar substrate with a photoresist layer of an epoxy-based negative photoresist;
   - soft baking the photoresist layer;
   - performing a full depth exposure with UV light through a first mask, thereby defining a first latent image of a vertical support structure extending over the full depth of the photoresist layer;
   - performing a partial depth exposure with UV light through a second mask, thereby defining a second latent image of a lateral structure in a top portion of the photoresist layer;
   - wherein the full depth exposure and the partial depth exposure are aligned to ensure that the first and second latent images are connected to each other;
   - post-exposure baking the photoresist layer, thereby creating a post-exposure bake latent image from the combined latent images; and
   - developing the microscale patterned resist template as a free-standing structure of cross-linked resist with lateral hanging structures that are supported by vertical support structures at a free height above the substrate;

   characterized in that

   soft baking is performed at temperatures not exceeding 70 °C.

2. Method according to claim 1, wherein the epoxy-based negative photoresist is an SU-8 based photoresist.

3. Method according to claim 1 or claim 2, wherein a soft baking time is adjusted to ensure a residual solvent concentration above 6%, or above 8%, or above 10%, or even above 15% by weight.

4. Method according to any of the preceding claims, wherein the soft baking temperature is between 20 °C and 60 °C, preferably between 30 °C and 55 °C or about 50 °C.

5. Method according to any of the preceding claims, wherein post-exposure baking is performed at a temperature equal to or below the soft baking temperature.

6. Method according to any of the preceding claims, wherein vertical critical dimensions of the microscale patterned resist template produced by the method are less or equal to 40 microns, less or equal to 30 microns, less or equal to 20 microns, or even less or equal to 10 microns, wherein the vertical critical dimensions are one or more of the vertical free height between the substrate and the lateral hanging structures, and the vertical thickness of the lateral hanging structures.

7. Method according to any of the preceding claims, wherein lateral critical dimensions of the microscale patterned resist template are less or equal to 40 microns, less or equal to 30 microns, less or equal to 20 microns, or even less or equal to 10 microns, wherein the lateral critical dimensions are one or more of a lateral transverse dimension of the support structure, a lateral distance between adjacent support structures, and a lateral thickness of the lateral hanging structure.

8. Method according to any of the preceding claims, wherein a plurality of vertical support structures of the microscale patterned resist template are arranged in a periodic manner in at least one lateral direction.

9. Method according to any of the preceding claims, wherein the hanging structures are formed so as to connect neighbouring support structures.

10. Method according to any of the preceding claims, further comprising after performing the partial depth exposure of the photoresist layer and after post-exposure baking

- applying one or more further photoresist layers;
- soft baking each of the further photoresist layers; and
- performing on each of the further photoresist layers a partial depth exposure with UV light through respective further masks, thereby defining in each further

photoresist layer in a top portion thereof a respective latent image of a further lateral structure.

11. Method according to claim 10, wherein a full depth exposure is performed for each further photoresist layer.

12. Method according to claim 10, wherein a single full depth exposure is performed for a plurality of vertically adjacent layers at the same time.

13. Method according to any of the preceding claims, wherein full depth exposures and partial depth exposures are performed at the same UV wavelength, such as the i-line at 365 nm.

14. Method according to any one of claims 1-12, wherein full depth exposures are performed at a first UV wavelength, such as the i-line at 365 nm, and the partial depth exposures are performed at a second UV wavelength different from the first UV wavelength, wherein the absorption of UV light by the epoxy-based negative photoresist is higher at the second UV wavelength as compared to the first UV wavelength.

15. Method of producing a three-dimensional microscale patterned resist template for a pyrolysed carbon microelectrode structure by means of UV-lithography, the method comprising:

- coating a planar substrate with a first photoresist layer of a first epoxy-based negative photoresist;
- soft baking the first photoresist layer;
- performing a full depth exposure with UV light through a first mask, thereby defining a first latent image of a vertical support structure extending over the full depth of the first photoresist layer;
- post-exposure baking the first photoresist layer;
- coating the first photoresist layer with a second photoresist layer of a second epoxy-based negative photoresist different from the first epoxy-based negative photoresist;
- soft baking the second photoresist layer;

- performing a partial depth exposure with UV light through a second mask, thereby defining a second latent image of a lateral structure in the second photoresist layer; wherein the full depth exposure and the partial depth exposure are arranged to ensure that the first and second latent images are connected to each other;
- wherein the partial depth exposure is performed using UV-light having a different spectral intensity distribution than the UV-light for the full depth exposure; wherein the second epoxy-based negative photoresist is more sensitive to the UV-light of the partial depth exposure than the first epoxy-based negative photoresist;
- post-exposure baking the second photoresist layer; and
- developing the microscale patterned resist template as a free-standing structure of cross-linked resist with lateral hanging structures that are supported by vertical support structures at a free height above the substrate;
- wherein soft baking is performed at temperatures not exceeding 70 °C.

16. Method according to claim 15, wherein the first epoxy-based negative photoresist is adapted for cross-linking with a maximum sensitivity to UV-light in a first range at wavelengths below 380nm, such as between 350nm and 380nm.

17. Method according to claim 15 or claim 16, wherein the second epoxy-based negative photoresist is adapted for cross-linking with a maximum sensitivity to UV-light in a second range at wavelengths above 380nm, such as between 380nm and 420nm.

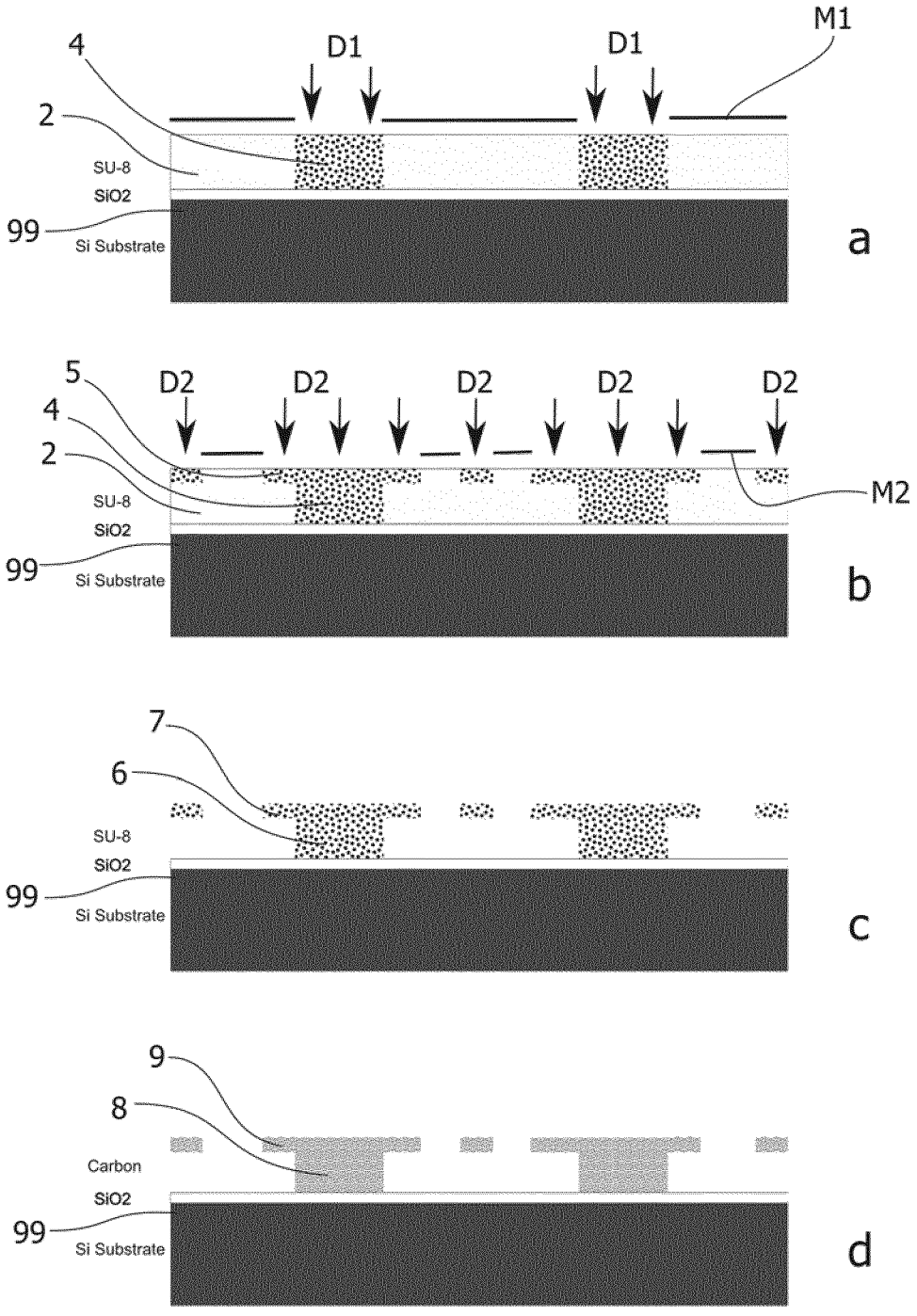
18. Method according to any of the claims 15-17, wherein the first epoxy-based negative photoresist is an SU-8 based photoresist.

19. Method according to any of the claims 15-18, wherein the soft baking temperature is between 20 °C and 60 °C, preferably between 30 °C and 55 °C or about 50 °C.

20. Method according to any of the claims 15-19, wherein post-exposure baking is performed at a temperature equal to or below the soft baking temperature.

21. Method of producing a pyrolysed carbon microelectrode structure on a substrate, the method comprising
- providing a microscale patterned resist template by a method according to any one of claims 1-20; and
- pyrolysing the microscale patterned resist template to obtain a pyrolysed carbon microelectrode structure with one or more laterally extending hanging portions suspended by one or more vertical support structures at a height above the substrate.

22. Microfluidic electrochemical cell comprising at least a working electrode, a counter electrode, and a reference electrode, or a working electrode and a counter electrode, wherein the working electrode comprises a truly three-dimensional microelectrode structure of pyrolysed carbon, wherein the truly three-dimensional microelectrode of pyrolysed carbon is fabricated by the method according to claim 21.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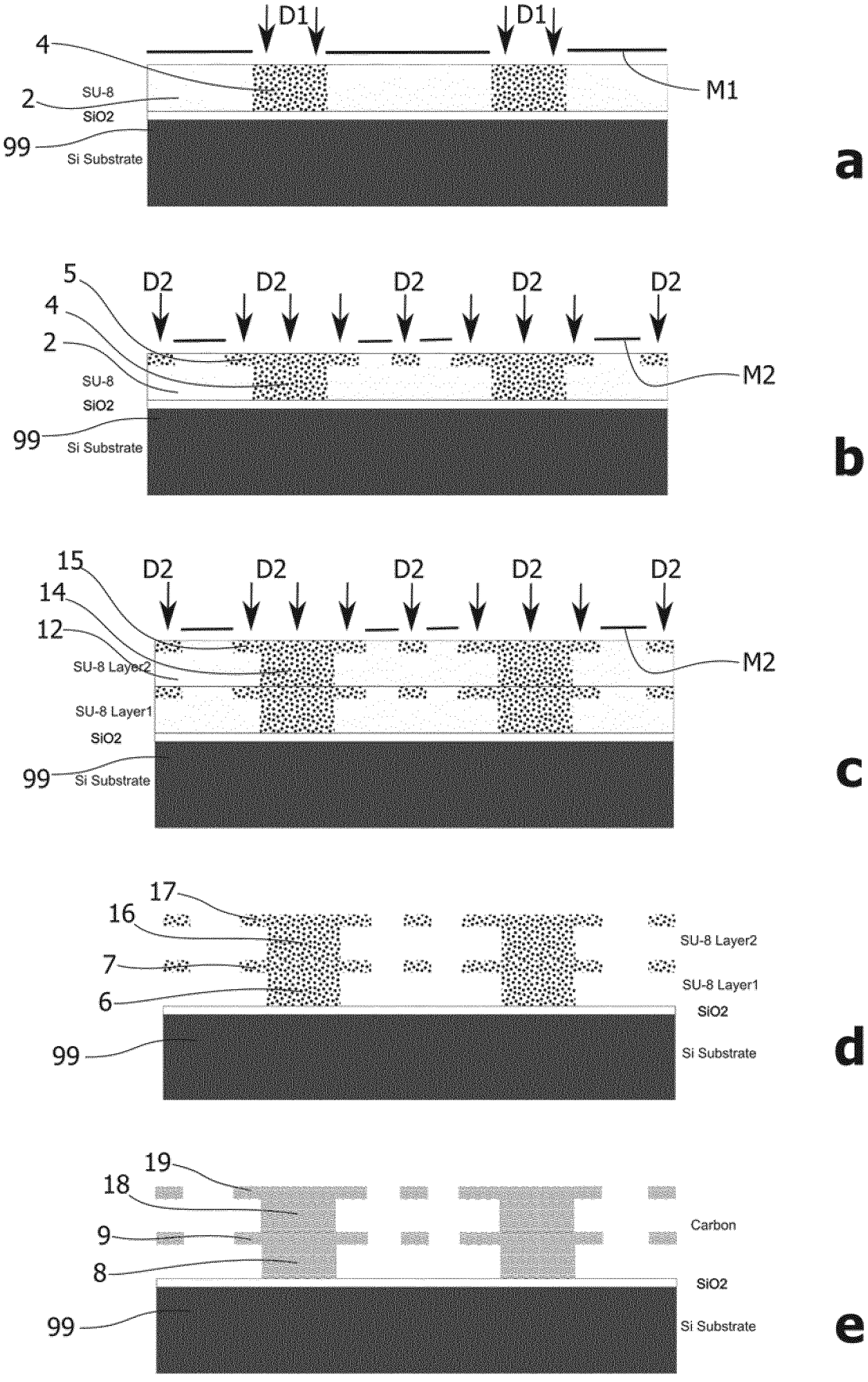
D1
D1
2
SU-8
SiO2
99
Si Substrate
a
5
D2
D2
D2
D2
D2
4
2
M2
SU-8
SiO2
99
Si Substrate
b
7
6
SU-8
SiO2
99
Si Substrate
c
9
8
Carbon
SiO2
99
Si Substrate
d

Fig. 1


D1
D1
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2
SU-8
SiO2
99
Si Substrate
M1
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D2
D2
D2
D2
D2
4
2
SU-8
SiO2
99
Si Substrate
M2
b
15
D2
D2
D2
D2
D2
14
12
SU-8 Layer2
SU-8 Layer1
SiO2
99
Si Substrate
M2
c
17
16
7
6
99
SU-8 Layer2
SU-8 Layer1
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Si Substrate
d
19
18
9
8
99
Carbon
SiO2
Si Substrate
e

Fig. 2

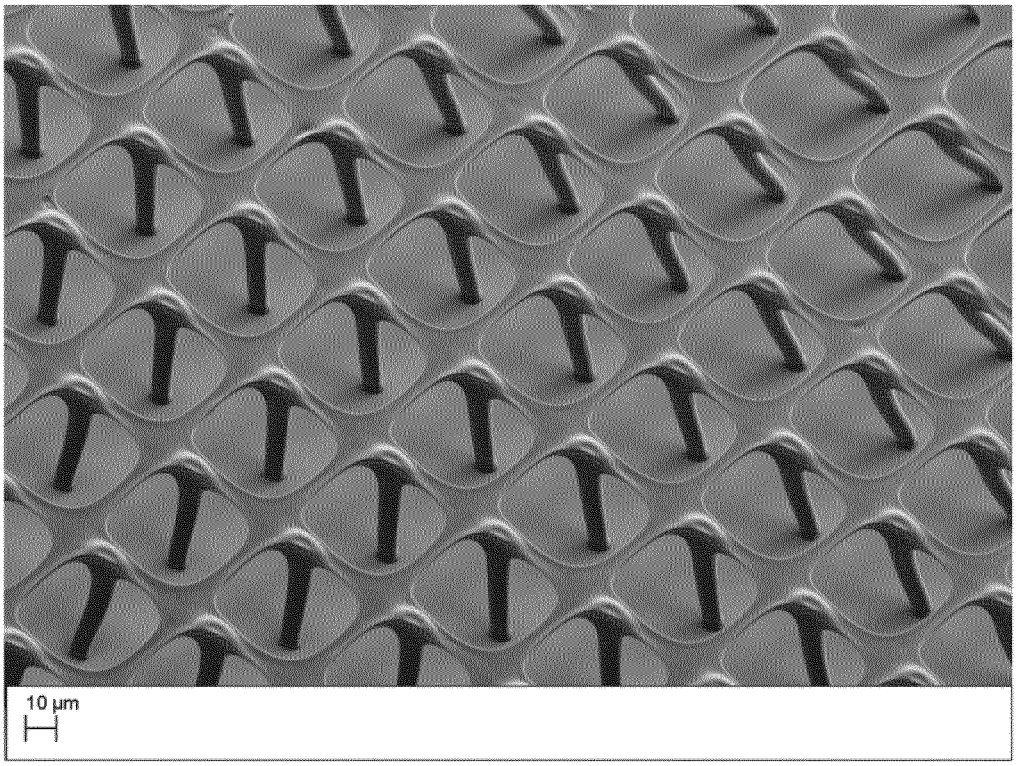

Fig. 3

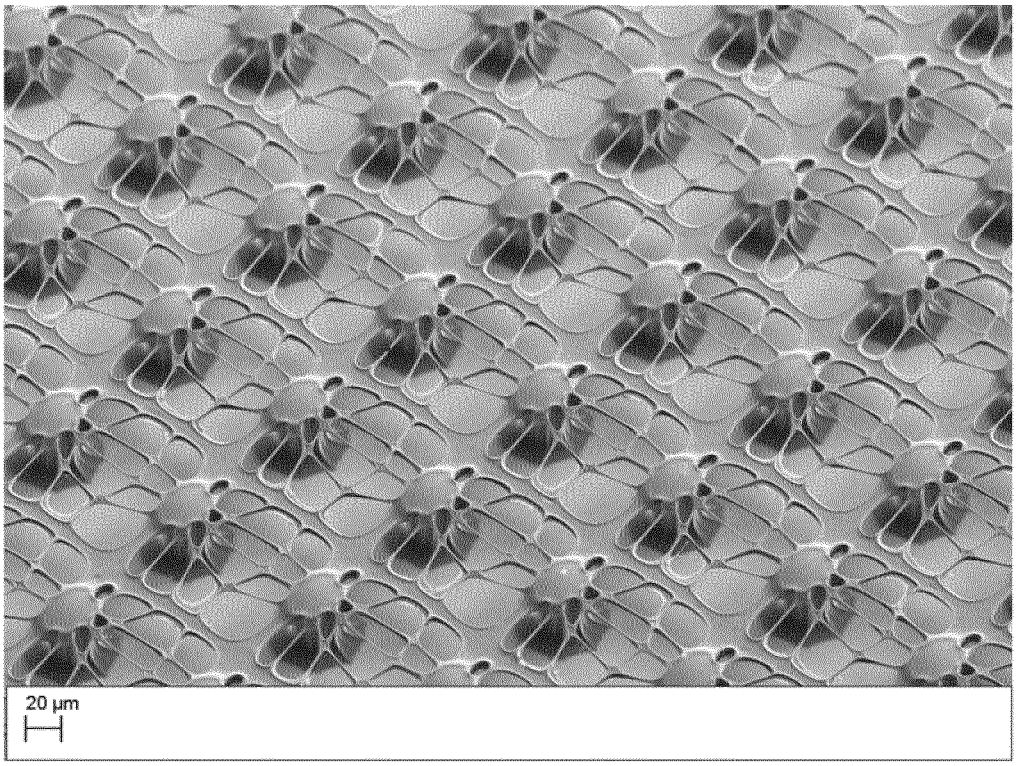

Fig. 4

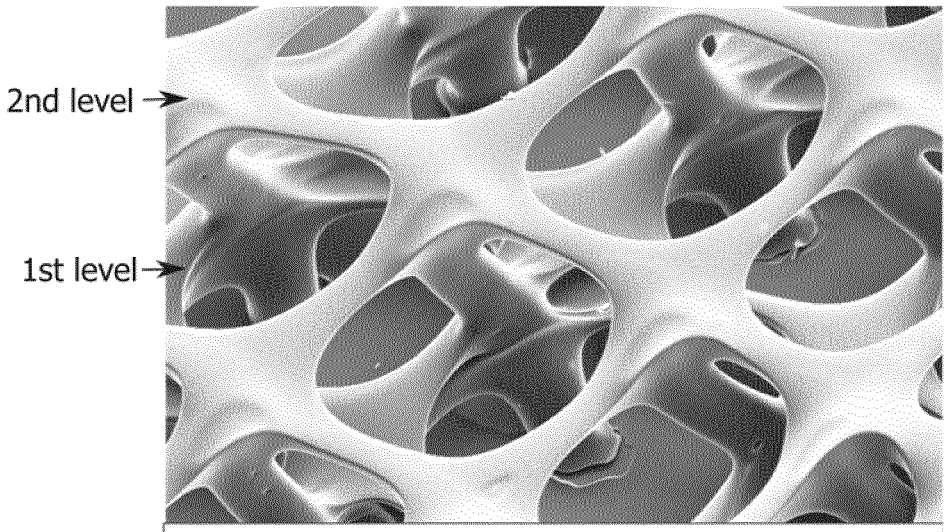

Fig. 5

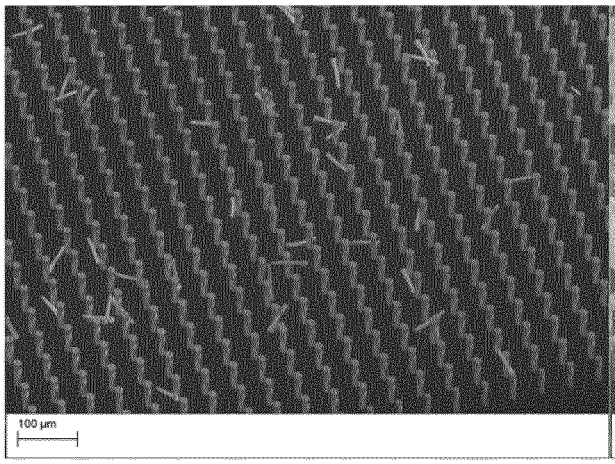

Fig. 6a

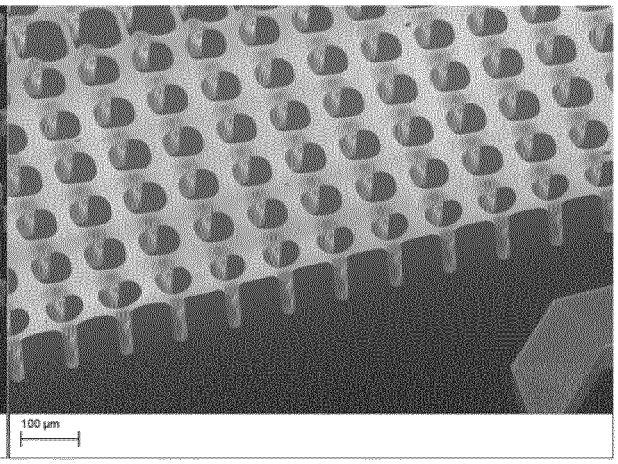

Fig. 6b

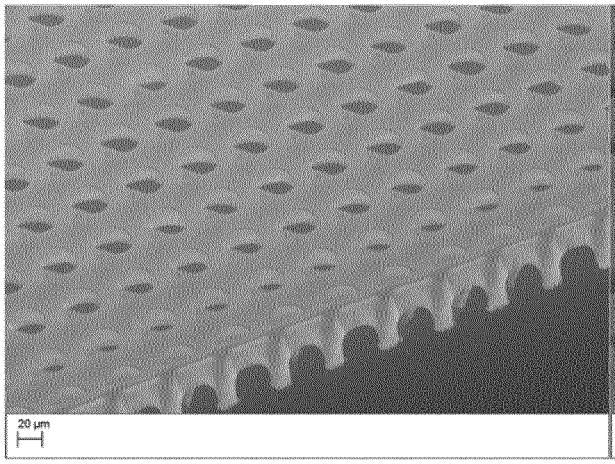

Fig. 6c

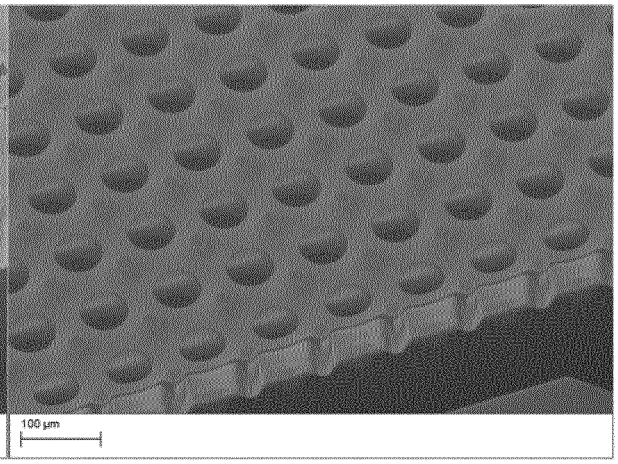

Fig. 6d

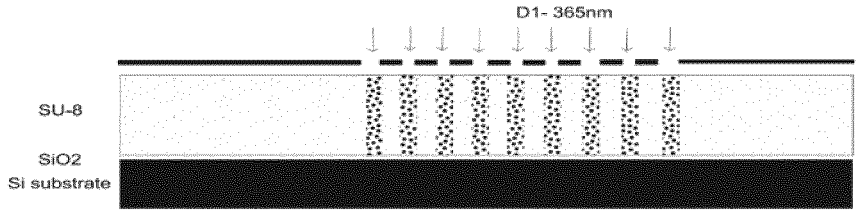

Fig. 7a

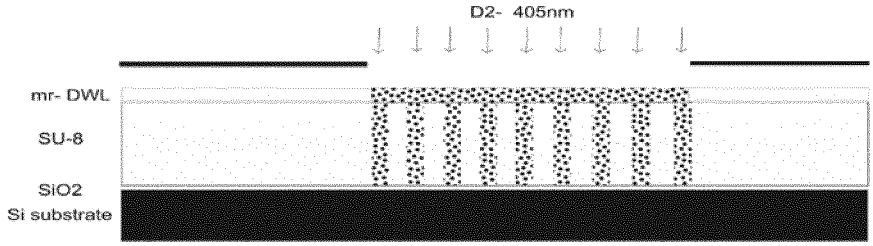

Fig. 7b

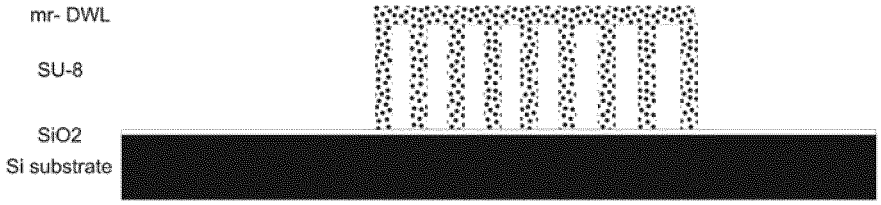

Fig. 7c

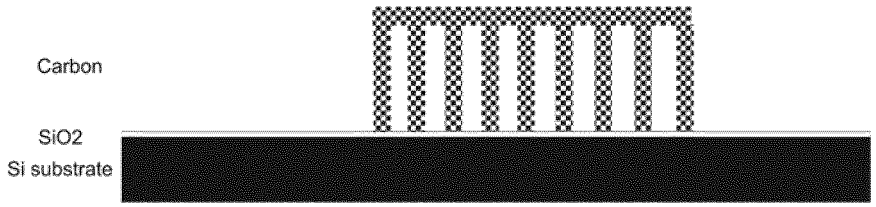

Fig. 7d

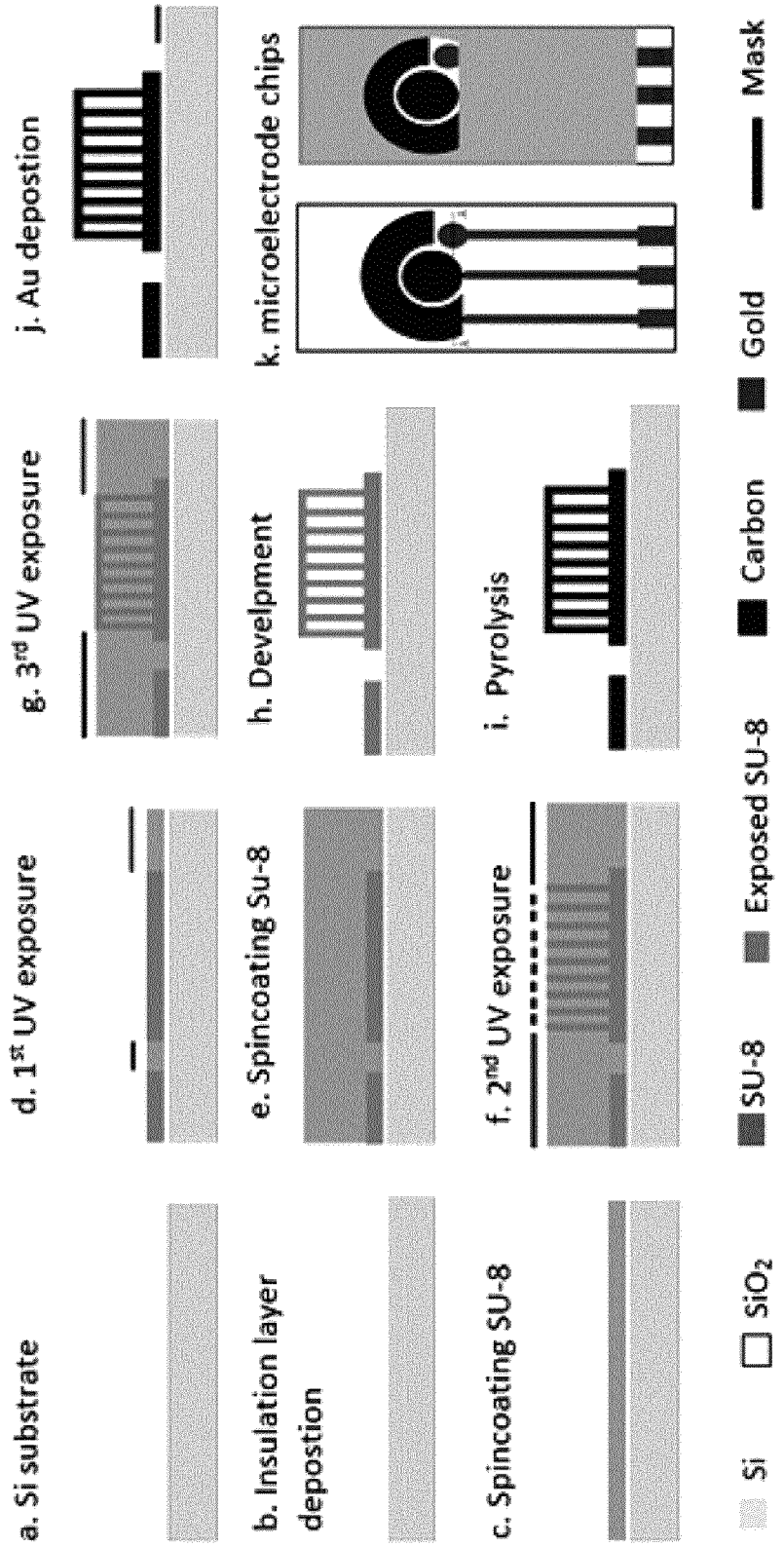
a. Si substrate
b. Insulation layer depostion
c. Spincoating SU-8
d. 1st UV exposure
e. Spincoating Su-8
f. 2nd UV exposure
g. 3rd UV exposure
h. Develpment
i. Pyrolysis
j. Au depostion
k. microelectrode chips
Si
SiO2
SU-8
Exposed SU-8
Carbon
Gold
Mask

Fig. 8

INTERNATIONAL SEARCH REPORT

International application No
PCT/EP2016/072388

A. CLASSIFICATION OF SUBJECT MATTER
INV. G03F7/00 G03F7/20 G03F7/213 G03F7/38
ADD.

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
G03F

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
EPO-Internal, WPI Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	JUNG A LEE ET AL: "Fabrication and characterization of freestanding 3D carbon microstructures using multi-exposures and resist pyrolysis", JOURNAL OF MICROMECHANICS & MICROENGINEERING, vol. 18, no. 3, 24 January 2008 (2008-01-24), page 035012, XP055262370, GB ISSN: 0960-1317, DOI: 10.1088/0960-1317/18/3/035012 cited in the application	22
A	page 4; figure 2 page 3, paragraph 2	1-21

-/--

[X] Further documents are listed in the continuation of Box C.

[X] See patent family annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier application or patent but published on or after the international filing date

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"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
24 October 2016	03/11/2016

Name and mailing address of the ISA/	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Fax: (+31-70) 340-3016	Rández García, J

1

Form PCT/ISA/210 (second sheet) (April 2005)

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	MALLADI K ET AL: "Fabrication of suspended carbon microstructures by e-beam writer and pyrolysis", CARBON, ELSEVIER, OXFORD, GB, vol. 44, no. 13, 22 June 2006 (2006-06-22) , pages 2602-2607, XP025010712, ISSN: 0008-6223, DOI: 10.1016/J.CARBON.2006.04.039 [retrieved on 2006-11-01] cited in the application	22
A	Section 2; figure 2	1-21

X	WANG C ET AL: "From MEMS to NEMS with carbon", BIOSENSORS AND BIOELECTRONICS, ELSEVIER BV, NL, vol. 20, no. 10, 15 April 2005 (2005-04-15), pages 2181-2187, XP027619567, ISSN: 0956-5663 [retrieved on 2005-04-15] cited in the application	22
A	Section 2; figure 3	1-21

X	US 2003/215753 A1 (TSENG FAN-GANG [TW] ET AL) 20 November 2003 (2003-11-20)	22
A	claims 1,3,8; figures 5a-5f	1-21

A	US 8 349 547 B1 (BURCKEL DAVID BRUCE [US] ET AL) 8 January 2013 (2013-01-08) cited in the application the whole document	1-21

A	YEONGJIN LIM ET AL: "Fabrication and application of a stacked carbon electrode set including a suspended mesh made of nanowires and a substrate-bound planar electrode toward for an electrochemical/biosensor platform", SENSORS AND ACTUATORS B: CHEMICAL: INTERNATIONAL JOURNAL DEVOTED TO RESEARCH AND DEVELOPMENT OF PHYSICAL AND CHEMICAL TRANSDUCERS, vol. 192, 27 November 2013 (2013-11-27), pages 796-803, XP055262334, CH ISSN: 0925-4005, DOI: 10.1016/j.snb.2013.11.065 cited in the application the whole document	1-21

1

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No
PCT/EP2016/072388

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
US 2003215753 A1	20-11-2003	TW 593128 B	21-06-2004
		US 2003215753 A1	20-11-2003
US 8349547 B1	08-01-2013	NONE	